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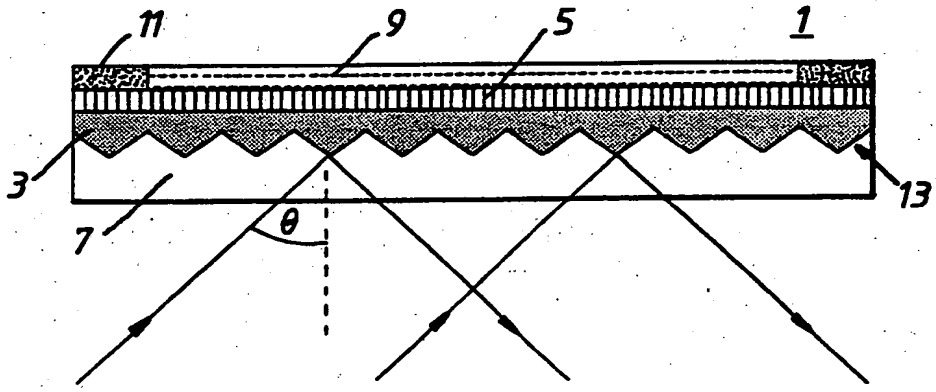

(57) Abstract

An optical biosensor is described in which a dielectric resonant cavity is formed of an ptically dense dielectric body (3 bounded on one of its plane faces by a medium (7) of lower refractive index material, and bounded on the other one of its plane faces by an organic coating (5) also of lower refractive index material. The coating (5) is sensitised to a specific assay species and is exposed to a test fluid sample (9) held within a container (11) that is adjacent to the coating (5). Coupling of light of resonant wavelength is facilitated by an optical grating (13) located either at the interface between the dielectric body (3) and the medium (7) or at the interface between the dielectric body (3) and the sensitised coating (5). The optical grating (13) may be embossed upon or engraved in the dielectric body (3). The dielectric body (3) and the medium (7) may be of nitrogen doped silica and silica, respectively. The medium (7) may be replaced by a multi-layer dielectric stack.

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IMPROVEMENTS IN OR RELATING TO OPTICAL BIOSENSORS

TECHNICAL FIELD

The present invention concerns optical biosensors for detecting and or monitoring or qualifying the presence and or behaviour of specific assay molecular species in test fluid samples. The invention has application to for example immunoassay i.e. the detection of antibodies, antigens or hormones in blood samples; pollution monitoring; and to the monitoring of clinical diagnostic reactions which may involve for example enzymes and the like.

BACKGROUND ART

In a recent article entitled "Detection of Antibody Antigen Reactions at a Glass Liquid Interface as a Novel Optical Immunoassay Concept, (1984)", R. M. Sutherland et al (Proceedings of 2nd Optical Fibre Conference (Stuttgart 1984), page 75) describe a biosensor wherein an antibody species is covalantly immobilised onto the surface of a planar or fibre optic waveguide. The reaction of immobilised antibody with antigen in sample solution is detected using the evanescent wave component of a light beam, totally internally reflected many times within the waveguide. The evanescent wave has a characteristic penetration depth of a fraction of a wavelength into the aqueous phase thus optically interacting with substances bound to or very close to the interface and only minimally with the bulk solution.

Reference is also made to our United Kingdom Patent Application GB. 2156970A published 16th October 1985, which discloses an optic waveguide biosensor and a similar technique.

Improved biosensor constructions which also rely on evanescent coupling with a sensitised layer are described in our co-pending United Kingdom Patent Application Nos. 2173895A (published 22nd October 1986) and 2174802A (published 12th November 1986). In both of these, resonant phenomena are exploited to provide signal enhancement. In the first of these a metallic medium is employed to produce high intensity well confined evanescent modes. The second of these concerns a resonant mirror structure in which a dielectric resonance is exploited. Specifically the latter structure comprises an optically dense body having on one of its surfaces a sensitised coating which acts as one mirror of the resonant cavity, and having at its other surface a partial mirror provided by a low index film or layered dielectric mirror sandwiched between the body and a coupling prism. It is a disadvantage that this latter structure is of somewhat bulky construction and assembly is demanding.

DISCLOSURE OF THE INVENTION

The present invention is intended to provide a biosensor that is of compact construction and which can be easily fabricated in large quantities and with the attendant economies of scale therefore can be manufactured at a relatively low cost and thus can be considered disposable.

In accordance with the present invention thus there is provided an optical biosensor comprising;

a test sample container;

a dielectric resonant cavity, mounted adjacent to the test sample container, and formed of a body of optically dense dielectric material bounded at each of its opposite principal plane faces by media of lower refractive index, one of these media being a coating sensitised for a specific assay species, this coating being located immediately adjacent to the test sample container and exposed for contacting a test sample fluid to be contained therein; wherein an optical grating is provided at one of the principal plane faces of the dielectric body for facilitating optical coupling between an optical source and an optical detector external thereto.

In the aforesaid construction the optical grating may be embossed upon or engraved in the dielectric body and may be provided either at the interface between the dielectric body and the sensitised coating or between the dielectric body and the other lower refractive index medium. In each case the grating provides a means of coupling light from an external light source into the resonant cavity thus formed and of coupling light from the resonant cavity onto an external detector.

It will be appreciated that in this manner a particularly compact construction may be manufactured.

The optical grating serves a dual purpose. Firstly it serves as a means of coupling light into the resonant cavity formed by the dielectric body, the sensitised layer and the other lower refractive index medium. Secondly it also will reflect and out-couple light and

it is this light that would be monitored by an external detector. Changes in the phase and amplitude of this light are most marked around the coupling angle. Reactions between a test sample and the sensitised layer change the resonant mode wave vector K_m and result in changes in the amplitude and phase of the reflected out-coupled beam. The incident beam may be directed at one or at a range of angles to the biosensor and the reflected out-coupled beam may be monitored at one or at a range of angles.

The wavelength of light used can range from the ultra-violet through the visible to the infra-red the dimensions of the biosensor components being scaled accordingly.

Various optical materials can be used to construct the biosensor depending on the design wavelengths. An example of a system for use over a large wavelength range is silica for one of the lower refractive index media, and nitrogen doped silica for the optically dense material of the dielectric body.

The optical grating may be formed using a number of techniques, such as etching or embossing. However, the embossing technique lends itself to high volume production of devices.

BRIEF INTRODUCTION OF THE DRAWINGS

In the drawings accompanying this specification;

Figure 1 is a cross-section of a biosensor constructed in accord with this invention and in which an optical grating is formed at the interface between the optically dense dielectric body and a single layer lower refractive index medium on the plane face of the body opposite to the sensitised coating;

Figure 2 is a cross-section of an alternative construction of biosensor also in accord with the present invention and in which the optical grating is formed at the interface between the optically dense dielectric body and the sensitised coating; and,

Figure 3 is a cross-section view of alternative construction of biosensor similar to that shown in the preceding Figure 2 but in which the single layer lower refractive index medium is replaced by a layered dielectric stack.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

So that this invention may be better understood, embodiments of this invention will now be described and reference will be made to the drawings. The description that follows is given by way of example only.

A resonant grating biosensor 1 is shown in Figure 1. This is comprised of an optically dense body 3 bounded on each of its principal plane faces by lower refractive index media 5 and 7. One of these media, media 5, has the form of an organic coating which is sensitised to a specific assay species which it is intended to detect. The sensitised coating 5, for example, may consist in a layer of monoclonal antibodies (refractive index n ≈ 1.4 to 1.5). The sensitised coating 5 is exposed to a test fluid sample 9 which is contained within a test sample container 11 the walls of which are shown in the figure. This arrangement is modified by the provision of an optical grating 13 which, as shown in this figure, is located at the interface between the optically dense body 3 and the lower refractive index medium 7. This optical grating 13 may be formed in

either the optically dense body 3 or the lower refractive index medium 7 and may be formed for example by engraving. Alternatively and in preference to this however, the optical grating 13 may be formed upon the principal plane face of the optically dense body 3 or upon the lower refractive index medium 7 and may be produced for example by an embossing technique during the course of manufacture.

The precise value for the thickness of the optically dense body will depend on the design wavelength of light used, the refractive index of the optically dense body material 3, the refractive index of the lower refractive index medium 7 (from which medium 7 light is incident upon the grating), and the pitch of the optical grating 13. This value can be calculated using the techniques for reflection from multi-layer structures, as outlined in "Principle of Optics", M. Born and E. Wolf, sixth edition (1980) Pergamon Press, page 66 ff. Typical values of optical thickness range from one half of one wavelength to one wavelength (i.e. the product of the refractive index and real thickness, $nt \approx 0.5 - 1.0\ \lambda$).

Grating structures are described for example in the following article, A. Yariv, IEEE Journal Quantum Electronics, Vol. QE-9, No. 9, September 1973. page 919. The pitch of the grating must satisfy the following equation;

$$\beta = k_o\ n \sin\theta \pm 2\pi m/\Lambda$$

where β is the mode constant of optically dense body material,

k_o is the light propagation constant ($= 2\pi/\Lambda$),

n is the refractive index of the lower refractive index medium from which light is instant upon the grating,

θ is the incident angle of the incident light beam subtended at the grating in the medium of refractive index n,

m = 1, 2, 3 ....., &

Λ is the pitch of the grating.

For light of the He-Ne wavelength, 632.8 nm, at an incident angle of around 60°, the pitch of the grating is typically one micron ($\Lambda \approx 1\mu m$).

The depth of the grating is dependent upon coupling efficiency and is typically of the order 1000Å.

The grating profile is secondary to its periodicity, and although the profile may be controlled, for example to blaze the grating for a particular angle, this profile would usually be chosen to be approximately sinusoidal.

Although a number of techniques for thin film deposition may be used to fabricate the resonant grating structure, in this example the structure is formed using silica and nitrogen doped silica respectively which are deposited by the process of magnetron sputtering. In the visible spectrum, these materials have refractive indices of 1.46 and 1.5 to 1.55, respectively. The latter index value is dependant upon the content of nitrogen dopant. Here the target is of the pure base material, in this case silica, and is sputtered onto a sample substrate in an inert argon atmosphere. Typical sputtering powers are 200 watts. To vary the refractive index of the silica, a second gas, nitrogen, is mixed with the argon, in proportions of around 30% active to inert gas. Sputtering times are typically half an hour.

An alternative arrangement is shown in Figure 2. In the biosensor 15 shown the optical grating 13 is provided at the interface between the optically dense body 3 and the sensitised coating 5. In this case also the optical grating 13 may be embossed upon a principal plane surface of the optically dense body 3 or it may be a relief grating engraved in this body 3.

In Figure 3 a similar biosensor arrangement 17 is shown but in this construction the single layer lower refractive index medium 7 has been replaced by a multi-layer dielectric stack 19. This multilayer stack 19 consists of alternate layers of quarter wave thickness of high and low refractive index materials, e.g. zinc sulphide (n = 2.3) and magnesium fluoride (n = 1.4). The number of layers adopted is determined in accordance with the reflectivity required (See "Principles of Optics", M. Born & E. Wolf, Sixth Edition (1980) Pergamon Press, page 66 ff).

CLAIMS:-

WHAT I/WE CLAIM IS:-

1. An optical biosensor comprising:

a test sample container;

a dielectric resonant cavity, mounted adjacent to the test sample container, and formed of a body of optically dense dielectric material bounded at each of its opposite principal plane faces by media of lower refractive index, one of these media being a coating sensitised for a specific assay species, this coating being located immediately adjacent to the test sample container and exposed for contacting a test sample fluid to be contained therein; wherein an optical grating is provided at one of the principal plane faces of the dielectric body for facilitating optical coupling between an optical source and an optical detector external thereto.

2. An optical biosensor, as claimed in claim 1, wherein the optical grating is embossed upon the said one of the principal plane faces of the dielectric body.

3. An optical biosensor, as claimed in either claims 1 or 2, wherein the optical grating is provided at an interface between the dielectric body and the sensitised coating.

4. An optical biosensor, as claimed in either claims 1 or 2, wherein the optical grating is provided at an interface between the dielectric body and that medium of lower refractive index that is opposite to the sensitised coating.

5. An optical biosensor, as claimed in any one of the preceding claims, wherein the dielectric body is of nitrogen doped silica and the

medium of lower refractive index, that is opposite to the sensitised coating, is of silica.

6. An optical biosensor, as claimed in any one of the preceding claims 1 to 4 wherein a multi-layer dielectric stack is provided in place of the medium of lower refractive index that is opposite to the sensitised coating.

7. An optical biosensor constructed, adapted and arranged to operate substantially as described hereinbefore with reference to and as shown in any one of Figure 1 to 3 of the accompanying drawings.

8. An optical biosensor system comprising in an operative combination:

an optical biosensor as claimed in any one of the preceding claims;

a light source arranged at a coupling angle relative to the dielectric resonant cavity to couple light of a resonant wavelength therein; and

a light detector arranged relative to the dielectric resonant cavity to receive light out-coupled therefrom.

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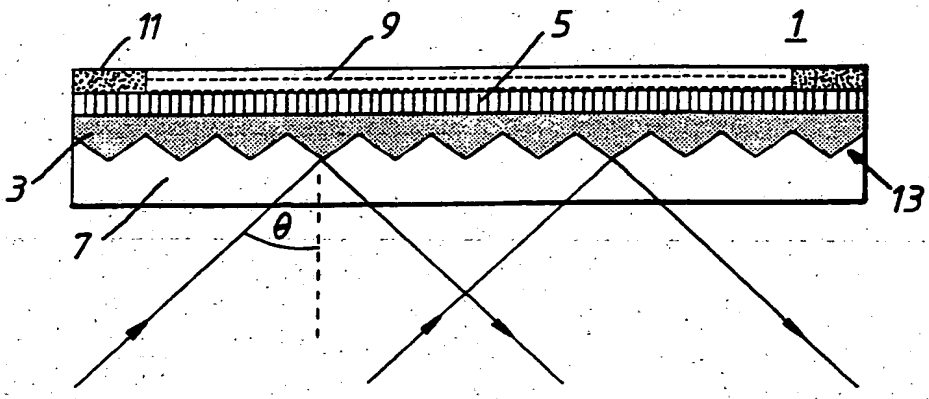

Fig.1.

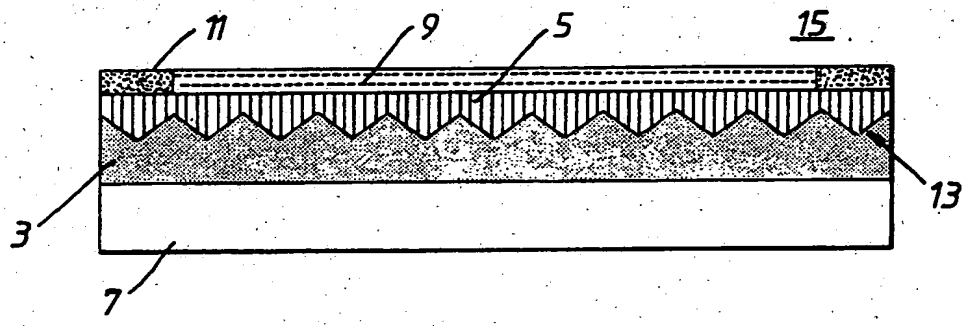

Fig.2.

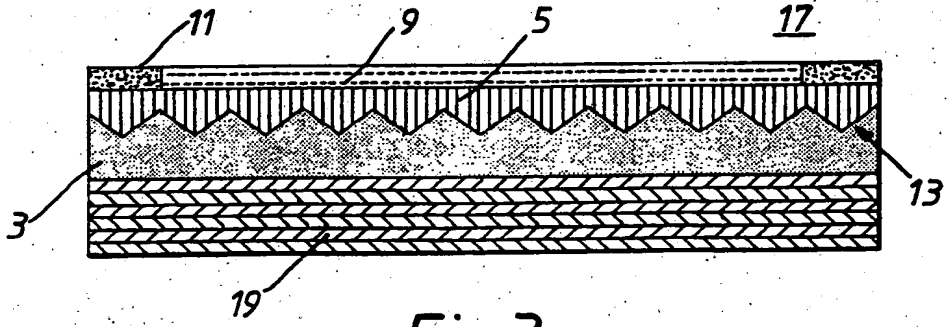

Fig.3.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/01461

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 33/53, 21/75

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
IPC5	G 01 N

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	Sensors and Actuators, Vol. 15, 1988, W Luhosz et al: "Sensitivity of integrated optical granting and prism couplers as (bio)chemical sensors ", see page 273 - page 284	1-10
A	EP, A2, 0257955 (THORN EMI PLC) 2 March 1988, see the whole document	1-10
A	GB, A, 2174802 (THE PLESSEY COMPANY PLC) 12 November 1986, see the whole document	1-10

* Special categories of cited documents: 10

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12th March 1990	02. 04. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 89/01461

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/02/90 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0257955	02/03/88	JP-A- 63075542	05/04/88
GB-A- 2174802	12/11/86	EP-A- 0205236	17/12/86
		JP-A- 61292044	22/12/86
		US-A- 4857273	15/08/89